SUPPLEMENTARY MATERIALS AND METHODS

Immunohistochemical staining and digital analysis

Primary antibodies used were polyclonal rabbit antibodies specific for p38, ERK, JNK (Cell Signaling, Beverly, MA) and murine monoclonal antibodies for phosphorylated (p)- p38, ERK, and JNK (Santa Cruz Biotechnology, Santa Cruz, CA). Sections were developed with goat anti-mouse or swine-anti-rabbit -horseradish peroxidase (HRP)-conjugated antibodies (Dako, Glostrup, Denmark), biotinylated tyramide and streptavidin-HRP, and aminoethylcarbazole (Sigma, St. Louis, MO).(33) Slides were counterstained with Mayer's hematoxylin, mounted and analyzed by digital image analysis in a blinded fashion using a Syndia algorithm on a Qwin-based analysis system (Leica, Cambridge, UK).(34) Expression and phosphorylation of proteins was calculated for each section as the median integrated optical density (IOD) per mm^2 tissue. Relative phosphorylation values were obtained by dividing IOD/mm^2 p-MAPK IOD by IOD/mm^2 total MAPK, normalized for tissue cellularity (nucleated cells/mm^2).

Isolation of synovial mRNA and analysis of gene expression

For analysis of synovial tissue gene expression, total RNA was isolated from biopsies of RA patients with self-limiting disease (n = 8) or persistent disease (non-erosive or erosive, n = 14) using RNA STAT-60^{TM} (Invitrogen, Breda, The Netherlands) according to the manufacturer's instructions. Subsequently, the total RNA fraction was cleaned using Rneasy spin columns (Qiagen, Venlo, The Netherlands) including a DNAse step to remove genomic DNA. Quantity and purity of the RNA was tested using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE). 250 ng of total RNA was reverse transcribed using an RT^2 First Strand Kit (SABiosciences) and expression of 84 genes involved in the regulation of cell adhesion and extracellular matrix was analyzed by quantitative (q)PCR. qPCR reactions were

performed on a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA) using an RT^2 Profiler™ PCR Array set (PAHS-013, SABiosciences) according to the manufacturer's instructions. Briefly, diluted cDNA was mixed thoroughly with RT^2 SybrGreen Rox qPCR Master Mix (SABiosciences) and 25 µl of the experimental cocktail was added to each well of the PCR array. After PCR amplification, threshold values were manually equalized for all samples and the threshold cycle (Ct) determined for each well. Relative gene expression was calculated for each gene using StepOne Software v2.1 (Applied Biosystems) and Microsoft Excel spreadsheet software (Microsoft, Redmond, WA), and expressed as the ratio between the gene of interest and Ribosomal protein L13a (RPL13A).Expression levels of genes measured by qPCR were analyzed by supervised hierarchical clustering and visualization by Treeview.